Cytokine Serum Level During Severe Sepsis in Human IL-6 as a Marker of Severity

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Forty critically ill surgical patients with documented infections were studied during their stay in an intensive care unit. Among these patients, 19 developed septic shock and 16 died, 9 of them from septic shock. Interleukin 1β (IL-1β), tumor necrosis factor (TNFα), and interleukin 6 (IL-6) were measured each day and every 1 or 2 hours when septic shock occurred. Although IL-1β was never found, TNFα was most often observed in the serum at a level under 100 pg/mL except during septic shock. During these acute episodes TNFα level reached several hundred pg/mL, but only for a few hours. In contrast, IL-6 was always increased in the serum of acutely ill patients (peak to 500,000 pg/mL). There was a direct correlation between IL-6 peak serum level and TNFα peak serum level during septic shock and between IL-6 serum level and temperature or C-reactive protein serum level. Moreover, IL-6 correlated well with APACHE II score, and the mortality rate increased significantly in the group of patients who presented with IL-6 serum level above 1000 pg/mL. Thus, IL-6 appears to be a good marker of severity during bacterial infection.

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INTERLEUKIN 1β (IL-1β) AND TUMOR NECROSIS FACTOR α (TNFα) are two cytokines primarily produced by activated macrophages in response to injury.[1] They mediate several systemic changes associated with trauma or infection such as fever, neutrophilia, and increased hepatic acute phase protein synthesis.[2] It is now thought that many of the consequences of bacterial sepsis are due to the release of these polypeptides. In particular, TNFα has been shown to be involved in septic shock.[3] When injected intravenously in animals, it produces the same effects as endotoxin[4,5]; passive immunization against TNFα also protects mice, rabbits, or baboons from the lethal effects of endotoxin or gram-negative bacteremia.[6-8] In humans, TNFα can be detected in the serum of volunteers after endotoxin injection.[9,10] During septic shock, high TNFα serum levels have been observed by many authors in severely ill pediatric or adult patients.[11-16] The role of IL-1β is less clear; like TNFα it can induced a shock state in rabbits,[17] and high peak levels were observed in purpura fulminans by Girardin et al.[12]

Interleukin 6 (IL-6) is another cytokine that appears to be the most efficient stimulator of the production by the liver of the acute phase proteins. It is probably a second messenger released by macrophages, endothelial cells, or fibroblast and other cells in response to IL-1β or TNFα.[18] High serum levels of IL-6 measured by bioassay have already been found during sepsis in humans.[19,20]

To document the involvement of IL-1β, TNFα, IL-6 in human infection, and in particular in septic shock, we investigated critically ill patients during the course of bacterial infections.

Materials and Methods

Patients

Forty critically ill patients with documented infections were studied during their stay in a surgical intensive care unit. All patients (or their relatives) gave us informed consent to draw blood and perform cytokines measurement. There were 20 intra-abdominal infections (12 with gram-negative bacteria [GNB], 2 gram-positive cocci, 2 anaerobic bacteria, and 4 with mixed flora), 5 mediastinitis (1 *Staphylococcus aureus,* 4 GNB), 3 bronchopneumonia (1 *S. aureus,* 2 GNB), 9 septicemia (1 *S. aureus,* 8 GNB), 2 vascular graft infections (1 *S. aureus,* 1 GNB) and 1 post-traumatic meningitis (1 GNB). For these patients, the usual clinical and biologic parameters, temperature, and leukocytosis were measured each day; serum C-re-

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active protein (CRP) was measured once a day, 5 days per week. APACHE II score, which is based on 12 bioclinical parameters in addition to age and chronic organ system failure, was recorded for each patient every day.[21]

During the course of infection, the diagnosis of septic shock was defined according to the following criteria: (1) systolic blood pressure less than 90 mmHg for more than 1 hour, or a fall of 50 mmHg in a previously hypertensive patient, (2) a rise in temperature to more than 39 C or a fall to less than 35 C, (3) a lactatemia equal or superior to 2.5 mmoles/L or oliguria of less than 20 mL/hour for 5 hours.

Sampling

Blood was drawn by venipuncture or using an indwelling arterial catheter into sterilized siliconed glass tubes (Becton Dickinson, Meylan-Cedex, France). The 40 patients were sampled once a day at 8:00 A.M. during 11.3 ± 9.7 days (range, 1 to 35); in addition, 20 of these patients were sampled 3.6 ± 1.8 times a day (2 to 8), every 1 or 2 hours as soon as they presented a septic shock state; septic shock was confirmed in 16 of these patients. Three other patients presented a septic shock during which only one sample was drawn. Clotting was obtained at room temperature. Blood samples then were centrifuged at 3000 rpm for 10 minutes, and separated serum was stored at −20 C until use.

Interleukin-6 was measured by a two-step immunoradiometric assay (IRMA) from Medgenix (Belgium). Briefly, the two-step IRMA is based on coated tube separation and on an oligoclonal system in which several monoclonal antibodies are directed against distinct epitopes of IL-6. The capture antibodies are attached to the lower and inner surface of plastic tubes. Two hundred microliters standard control solution and an equal volume of serum samples are each placed into duplicate coated tubes together with 50 μL of a special diluent. The tubes then are incubated for 16 to 20 hours at room temperature. The complete content is then aspirated and the tubes washed with Tween 20 (Merck, München, Germany) in distilled water (5%). After this procedure, 200 μL tracer, which is another monoclonal antibody labeled with I^{-125}, is added to the tubes for 2 hours. The tubes then are washed again and are counted in a gamma counter for 60 seconds. The IL-6 concentration of samples was determined by interpolation using a standard curve prepared at the same time. Interleukin-1β and TNFα were measured by a one-step IRMA using available kits from Medgenix (Belgium). The procedure of the one-step IRMA is the same as for the two-step IRMA, except that the standards or samples are incubated together with 50 μL tracer. The sensitivity of these IRMA assays are 5 pg/mL for TNFα and IL-6 and 4 pg/mL for IL-1β. In normal subjects, no IL-1β, no TNFα, and no IL-6 could be detected by IRMA.

C-reactive protein serum level was measured by nephelometry.

Statistics

Comparisons of biologic parameters or clinical scores between groups of patients were made using Student's test. To normalize the distribution of IL-6 and TNFα serum results, and to permit the use of the t test, we used the log of the serum level, which is comparable to the median. The relationships between biologic or clinical parameters were tested by linear regression using standard formulas and chi square was used to establish a correlation between IL-6 serum level and mortality rate. The level of significance was fixed at 5%.

Results

Among the studied patients, there were 29 men and 11 women, with a mean age of 60.5 ± 12.6 years (range, 29 to 82). The severity of their illness was assessed by APACHE II score, which, as a mean, was 21.4 ± 6.7 at day 1. The mean time of their stay in the intensive care unit (ICU) was 24 ± 6.7 days. Nineteen patients developed septic shock as defined above, and 16 patients died, 9 of them from septic shock. Shock was due to GNB in 16 cases, to *S. aureus* in 3. As shown in Table 1, APACHE II score at day 3 was statistically different between survivors and nonsurvivors, but it could not distinguish between patients with or without septic shock.

Interleukin-1β was never found in the serum of any patient, even if they were sampled hourly during a septic shock episode. Tumor necrosis factor-α serum levels were just above the detectable level in most patients. In patients without septic shock (n = 21), only 52% of the samples contained TNFα. In five of these patients, TNFα was never found, whereas six patients only showed serum levels above 100 pg/mL; the maximum observed, in a patient with peritonitis, was 255 pg/mL (Fig. 1C). In the four patients without septic shock who were sampled 4 times in a day, TNFα serum levels remained low and constant. Conversely, patients with septic shock (n = 19) showed a brief TNFα peak during the acute episode, with a rapid return to lower values as shown in Figure 1A and B. The mean peak value of TNFα during septic shock was 735.9 ± 873 pg/mL (range, 10 to 3348), whereas it was 67.1 ± 63.7 pg/mL in patients without septic shock ($p < 0.001$). Three of the nineteen patients with septic shock did not have high levels of TNFα in their serum (*i.e.*, <100 pg/mL); they were all sampled the day after the onset of shock. Figure 2 shows the negative correlation between the peaks of TNFα serum level and leukocyte count measured at the same time in each patient ($r = -0.54$, $p < 0.01$). The time during which TNFα could be found in the serum did not differ between survivors and nonsurvivors.

TABLE 1. *Cytokine Peak Serum Levels in Patients According to the Presence of Shock or the Occurrence of Death*

	Survivors	Nonsurvivors	Without Shock	With Shock
No. of patients	24	16	21	19
Age	58.3 ± 13.2	63.7 ± 11.6	60.8 ± 12.7	59.8 ± 13.5
APACHE II				
Day 1	20.2 ± 6.1	23.2 ± 7.3	19.9 ± 6.6	22.8 ± 6.7
Day 3	13.6 ± 4.6*	23.7 ± 6.5*	16.4 ± 6.3	18.5 ± 8.4
IL-1β peak serum level	0	0	0	0
TNF peak serum level (pg/mL)				
Median	93	120	57*	471*
Range	25–2286	10–3448	25–255	10–3348
IL-6 peak serum level (pg/mL)				
Median	1434	6598	788*	10049*
Range	200–217774	180–461850	180–3868	200–461850

* $p < 0.05$.

In contrast to IL-1β and TNFα, IL-6 serum levels were almost always above normal value during bacterial infection (Fig. 3). Only one patient was observed to have a normal value at the end of his stay in the ICU. Most values were between 10 and 1000 pg/mL, with some extreme levels reaching 500,000 pg/mL. The serum level of IL-6 seemed to be correlated with the severity of the illness. As shown in Table 1, there was a large difference between IL-6 peak levels observed in survivors and in nonsurvivors, as well as between patients with and without septic shock. Because of the high standard deviation, however, only the difference between septic shock and nonseptic shock was statistically significant. Furthermore, a good correlation could be observed between the log of IL-6 serum level and the APACHE II score obtained the day of the IL-6 peak as well as on day 3 or day 7 (Fig. 4). Several parameters also were found to be correlated with IL-6 serum levels: temperature, as shown by Figure 5, and CRP by Figure 6. This last figure was made taking the highest levels of IL-6 and the CRP serum level obtained on the

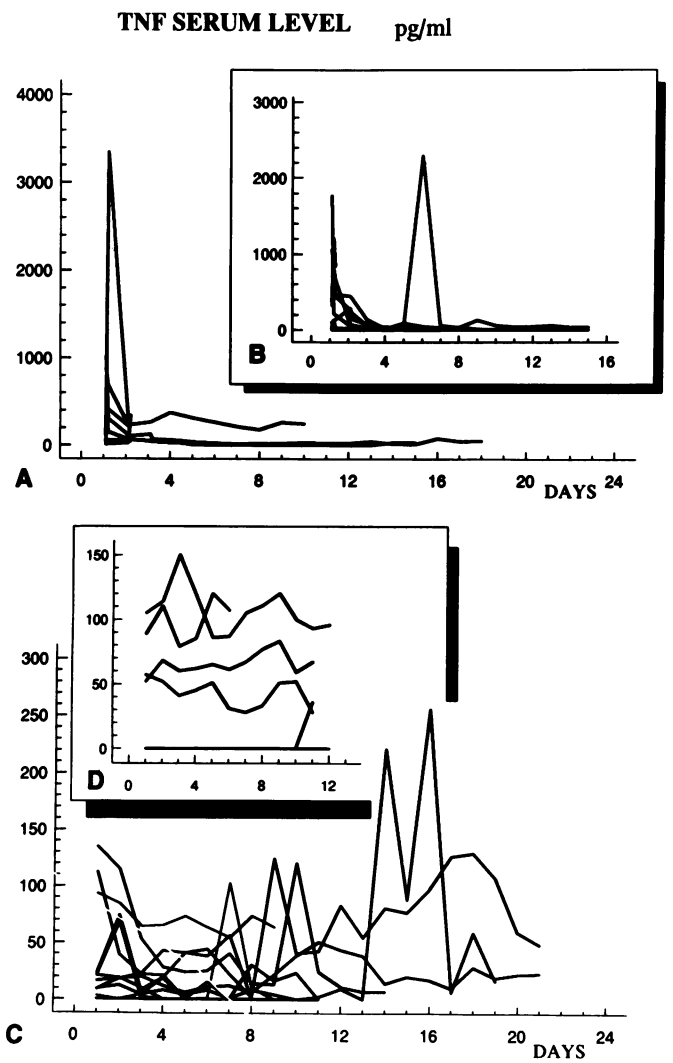

FIG. 1. Time course of TNFα serum levels during infection in patients with (A, nonsurvivors; B, survivors) and without septic shock (C, survivors; D, nonsurvivors).

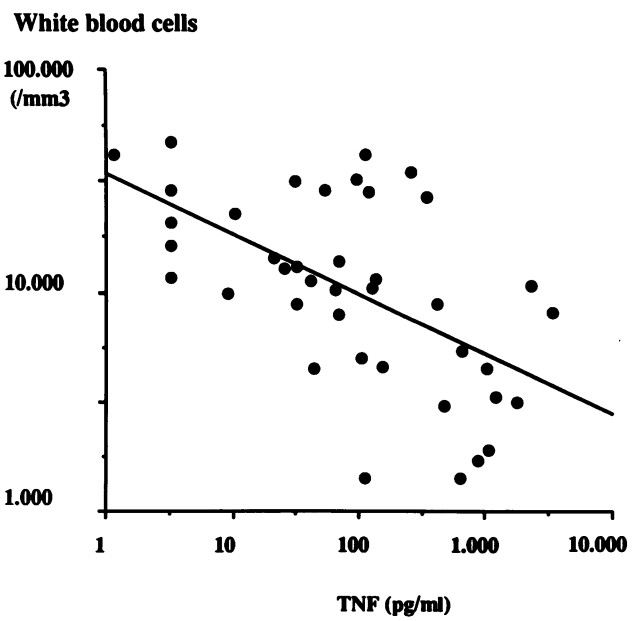

FIG. 2. Relationship between peak serum level of TNFα and white blood cell count measured at the same time ($r = -0.57$, $p < 0.001$).

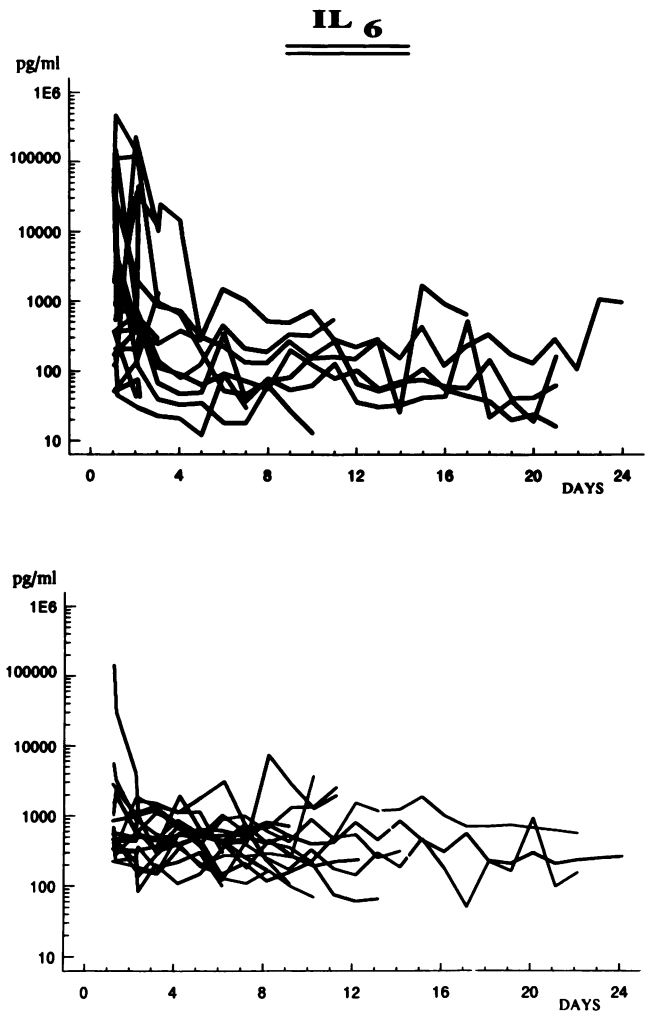

FIG. 3. Time course of IL-6 serum levels in patients (top) with and (bottom) without septic shock.

same day or the day after and the same parameters measured 1 week later (maximum 2 points per patient). Because the highest IL-6 values were observed during septic shock, a good correlation could be observed between IL-6 and TNFα ($p < 0.001$), and also a negative correlation between IL-6 and leukocytosis ($p < 0.001$). No correlation could be found, however, between IL-6 and lactate level or platelet count.

Discussion

Interleukin-1β could not be observed in our patients even at the time of septic shock. Several authors, however, have reported high serum or plasma levels of IL-1β during sepsis in animals or humans, using bioassay, radioimmunoassay, or enzyme-linked immunoassay.[12-15,19] The specificity of these assays may be questioned because some bioassays also may have been sensitive to IL-6,[22] and some immunoassays detected significant levels of IL-1β in healthy volunteers. Hesse et al.[9] described an increase in serum level of IL1β after the peak of TNFα, using a bioassay in normal volunteers challenged with endotoxin IV, whereas Michie et al.[10] did not observe any change in IL-1β measured by immunoassay. The high recovery of IL-1β added to either normal plasma or serum from septic patients suggests that the presence of serum inhibitors of IL-1β could not have impaired the detection of IL-1β in our patients. The involvement of circulating IL-1β during severe infection thus remained a matter of debate. Some recent studies, however, showing the protection by a receptor antagonist for IL-1 in septic shock model in animals support the role of IL-1.[23,24]

Tumor necrosis factor alpha is thought to be an early mediator of septic shock and has already been found by many authors in the serum of patients in septic shock. No longitudinal studies have yet been reported, however, except after burn injury.[25] This study confirms the involvement of TNFα in septic shock; high peak levels were observed in almost every episode of septic shock, and only during this acute event. Three patients were sampled too late after the onset of septic shock, and their TNFα serum levels were low, confirming the negative correlation between the peak serum level of TNFα and the time elapsed from the onset of shock already observed in a previous study.[13] In patients without shock or in the other patients beyond the septic shock episode, TNFα serum levels usually remained under 100 pg/mL. There was no correlation between the time during which TNFα could be found in the plasma and death. These data are consistent with those reported after endotoxin challenge in human volunteers, where a brief increase of TNFα serum level, negatively correlated with leukocyte count,[10] also was seen. This may be due to IL-8, however, another cytokine released after

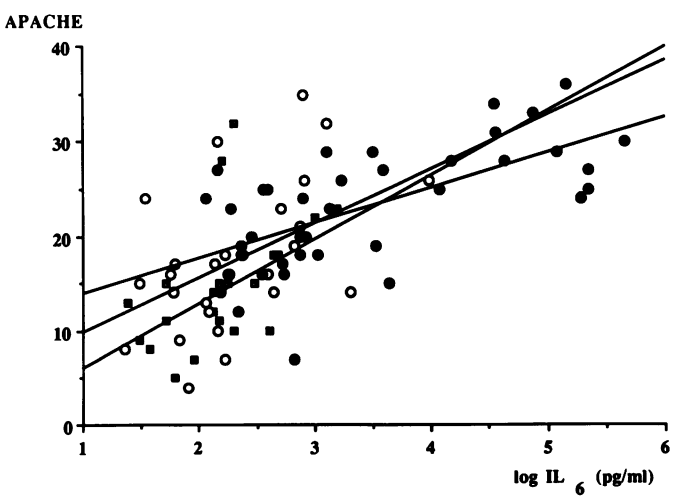

FIG. 4. Relationship between IL-6 serum level and APACHE II score. ●, peak value of IL-6; ○, IL-6 serum level at day 3; ⊡, IL-6 serum level at day 7 ($r = 0.63$, $p < 0.001$).

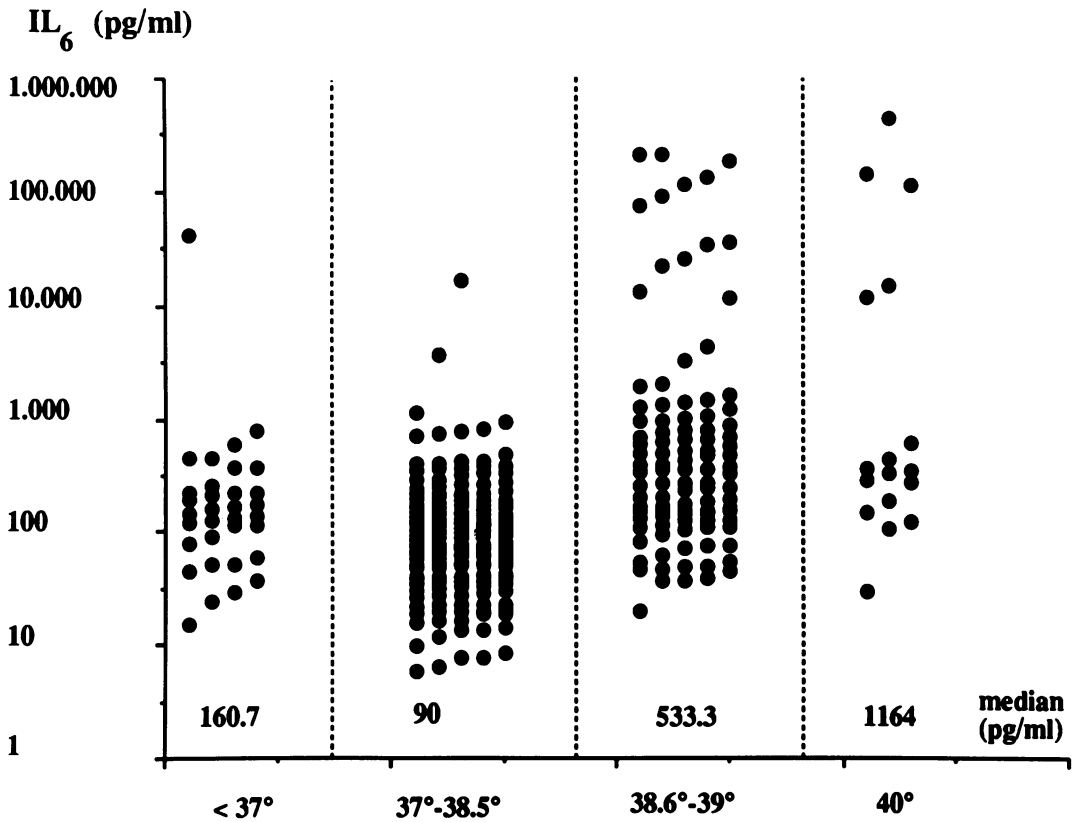

FIG. 5. Correlation of IL-6 serum level with maximum recorded temperature on the same day for the entire group of 40 patients. Median is the mean of the log of IL-6 serum level. The differences between these mean values are highly significant ($p < 0.001$).

TNF and IL-1 in the cytokine cascade.[26] During infection, when the leukocyte count was very high, we never observed high levels of TNFα.

The brief increase in TNFα serum level does not allow us to use this level as a prognostic threshold. As shown in Table 1, the prognosis depends on the APACHE II score rather than on a transient serum level, which can be missed.

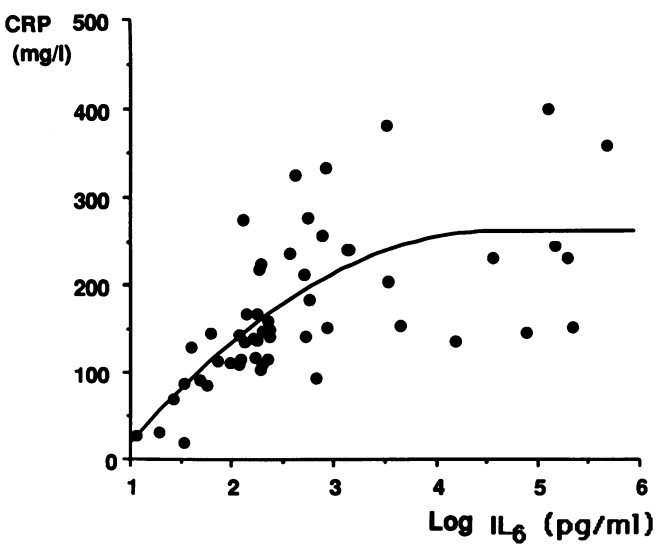

FIG. 6. Relationship of IL-6 and CRP serum levels (CRP measured within 24 hr of IL-6) on the day of peak IL-6 and 1 week later ($r = 0.66$, $p < 0.001$).

After IL-1β and TNFα, IL-6 is the third cytokine described to be involved in the acute phase response. Its multiple effects contribute to a coordinated response of the body to aggression: it acts to render T cells and hematopoietic cells responsive to their respective growth and differentiation factors, it initiates acute phase protein synthesis in the liver, and stimulates antibody production.[18] Its presence in the serum or plasma has already been reported in several circumstances, including burn injury,[27] after surgery,[28] during acute graft rejection,[29] in myeloma,[30] or during sepsis.[19,20] High plasma levels of IL-6, exceeding 10,000 pg/mL, have only been observed in septic patients using a bioassay.[20] After endotoxin injection in volunteers, a peak IL-6 plasma level of 25 ng/mL was observed after 2 to 4 hours, followed by a peak of CRP at 20 hours.[31] During acute graft rejection or after surgery, IL-6 levels were much lower, in the range of 100 to 1000 pg/mL.[29,30]

Our results confirm those of Hack et al.,[20] who found levels up to 150,000 pg/mL in septic patients at the time of admission to the ICU. Rather than the direct correlation

with mortality rate described by Hack et al., however, we found a significant correlation between the high serum levels of IL-6 and the illness severity as assessed by the APACHE II score. We found a level of 1000 pg/mL, above which the mortality rate significantly increased from 21% to more than 50%; this percentage did not change in patients demonstrating IL-6 levels higher than 100,000 pg/mL. The same can be seen with the APACHE II score, which could not differentiate between survivors and nonsurvivors on admission to the ICU. Thus, the outcome of patients does not depend on one measure only; it is rather the failure to respond to treatment, as shown by the APACHE II score on day 3, which is the valid predictor. The fact that IL-6 is really a marker of severity is emphasized by the correlation with APACHE II score which still exists at day 3 and day 7. One could say, however, that the APACHE II score is validated by such a correlation. As can be seen in Figure 4, however, several particular APACHE II scores were still high in spite of the decrease of their respective IL-6 levels, indicating that IL-6 seemed to return to baseline more rapidly than the correction of the clinical status as already mentioned by Hack et al.

Because IL-6 serum levels were very high during septic shock, it is not surprising to find a correlation between peak IL-6 and TNFα serum levels, as well as a negative correlation between peak IL-6 serum levels and leukocytosis. Tumor necrosis factor alpha peaks and the relative leukopenia were transitory events: therefore, this relationship appears to be ephemeral and is no longer evident after septic shock. More interesting is the relation between IL-6 and temperature, and especially CRP. Interleukin-6 is considered to be an endogenous pyrogen, and its serum level has been shown to be directly related to temperature after burn injury.[23] In this study, there was a considerable overlap of IL-6 serum levels between the different temperature intervals; the distribution, however, showed a statistically significant progressive increase of IL-6 as a function of recorded temperature. The relation to CRP, however, may be questioned because it was obtained using the peak serum level of IL-6 and the CRP serum level observed within 24 hours after this peak, as well as the same levels measured 1 week later in the same patients, when both decreased. It would have been preferable to document this relationship using different degrees of severity of infection and not the recovery phase after infection. This can be compared, however, with the results obtained after surgery: a direct relationship between the log of IL-6 serum level at 24 hours after surgery, and the peak of CRP serum level at 48 hours could be observed.[32] The plateau obtained for the response of CRP to very high levels of IL-6 with a relative dispersion of the results as shown in Figure 6 may be due to the fact that these points were obtained in severely ill patients with multiple system failure; thus, liver function and hepatic responsiveness to IL-6 may have been impaired. The involvement of IL-6 in the pathophysiology of sepsis and septic shock may be questioned in view of the relationship between serum level of IL-6 and the severity of the disease; it appears from animal studies, however, that this cytokine functions as an alarm signal, leading to the recruitment of host defenses.[18] This study emphasizes the high magnitude of this signal.

In conclusion, although IL-1β could not be found in the serum of infected patients, TNFα was observed mainly as a brief pulse at the time of septic shock. Interleukin-6 was, in contrast, often found at a serum level correlated to the severity of the illness, as assessed by APACHE II score, and to the acute phase response, as shown by its relationship with temperature and CRP.

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